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Effects of Spearmint (/-Carvon) Oil and Methyl Salicylate Oil Emulsion on Anesthesia of Common Carp (*Cyprinus carpio* L., 1758)

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Abstract

The aim of this study was to identify the times of anesthetic induction and recovery in common carp (*Cyprinus carpio*) with average body weights of 11.59 ± 1.23 g, at water temperatures of 23°C that were exposed to the spearmint (/-Carvon) oil and methyl salicylate oil emulsion (CMSE). Common carp were placed in 1-L aquaria containing different concentrations of CMSE (263, 395, 526, 658 and 789 µl/L). The different treatments resulted in average induction and recovery times ranging from 80.33 ± 5.13d s to 305.67 ± 21.13 s and 87.67 ± 19.39 s to 194.33 ± 27.09 s, respectively. Induction and recovery time decreased significantly with increasing of the concentration of CMSE ($P<0.05$). Opercular rate firstly increased and then slowly decreased with increasing the concentration of anesthetic. Glucose levels were significantly affected by CMSE concentration ($P<0.05$). Lower levels of plasma glucose after anesthesia and recovery belong to treatment of 526 µl/L. A second experiment was conducted in which common carp were immersed in either 526 µl/L CMSE or 150 mg/L clove powder in order to compare them with each other. Induction was quicker in the CMSE group; however, recovery was quicker in the clove powder group. Also, lower levels of plasma glucose and opercular rate belong to CMSE. No mortality was observed in the study. Applications of CMSE in 526 µl/L level, seems to be suitable on anesthesia of common carp.

Keywords: Anesthesia; Induction time; Glucose; Spearmint; Common carp

Introduction

Fish used in farming or research are exposed to many situations that are stressful or painful to the animals, including handling [1], confinement, netting, weighing, vaccination, blood sampling [2,3], transport [4], and surgical procedures [5,6]. The stress response is an adaptive physiological function that responds to a perceived threat to homeostasis, which in the short term preserves the health and viability of the stressed individual [7]. However, when the stress is excessive in either intensity or duration, the stress response can result in undesirable consequences, such as illness and mortality [8,9]. The use of anesthetics and sedatives may be beneficial in fish transportation by reducing physical activity and fish stress during handling [10-12] and to reduce mortality when exposed to severe and repeated stressors [13]. Though anesthetics can be a valuable tool to ensure animal welfare during these events, these agents can also have unwanted side effects and should therefore be used with caution [14].

Choosing an anesthetic must be attributed to several characteristics including its efficacy, availability; cost-effectiveness; ease of use; nature of the study; and safety for the user including fish, humans and the environment [15-17].

To minimize the effects of stress on fish, investigators worldwide have begun to examine the use of natural products with anesthetic properties. Examinations of chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) and persian sturgeon (*Acipenser persicus*) anesthetized with clove oil [6,15,18,19] and silver catfish (*Rhamdia quelen*) anesthetized with essential oil (EO) of *Lippia alba* [20,21], *Ocimum gratissimum* [22], *Aloysia triphylla* [23], and *Hesperozygis ringens* [24] have led to the development of anesthesia protocols in which products extracted from natural sources were employed with efficacy to treat fish.

One of these potential fish anesthetic compounds is an emulsified mixture of two common human food substances: spearmint oil (/-carvone) and methyl salicylate. Spearmint (*Mentha spicata*) has been consumed by humans for millennia; it has been written into mythical and religious tales, and today mint is the third most popular flavor in the world behind vanilla (*Vanilla planifolia*) and citrus [25]. It is used in cuisine, candy, chewing gum, cosmetics, toothpaste, tobacco products, and pharmaceutical preparations [26]. Carvone is the component of the spearmint oil that gives it a minty fragrance and flavor. Caraway (*Carum carvi*) seeds, dill weed (*Anethum graveolens*), mandarin orange (*Citrus reticulate*), peels, peppermint (*Mentha piperita*), and many fruits contain mixtures of both enantiomers of carvone and related substances that give them their unique fragrances. *I*-Carvone can be biosynthesized from tangerine peels or extracted from spearmint plants [27]. *I*-Carvone's anesthetic effect on the peripheral and central nervous systems include central nervous system depression, antinociceptive effects, sedation, and anticonvulsant-like activity [28-30].

Methyl salicylate can be synthesized by esterifying salicylic acid with methanol, but for centuries it was distilled from twigs of sweet birch (*Betula lenta*) and eastern teaberry (*Gaultheria procumbens*). Methyl salicylate is purported to be a nonsteroidal anti-inflammatory drug with pharmacological effects comparable to salicylic acid [31,32].

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Therefore, the aim of this study was to examine the efficacy and determine the optimum concentration of Spearmint (*l*-Carvon) Oil and Methyl Salicylate Oil Emulsion (CMSE) as common carp anesthetic and compared with commonly fish anesthetic clove powder. The physiological response of plasma glucose was also subsequently investigated.

Materials and Methods

Animal

These experiments were conducted on common carp with a mean length of 9.8 ± 0.6 cm and weight of 11.59 ± 1.23 g. The experiment was carried out in 2 m3 tank. All of the fish were starved for 24 h prior to experiment [33]. Experiment was performed out at 23°C water temperature and in triplicate.

Immersion anesthetic formulation and preparation

Five different concentrations of CMSE (263, 395, 526, 658 and 789 μI/L) were chosen according to the pervious studies. The CMSE is composed of 28.4% *I*-carvone (pmentha- 6,8-dien-2-one), 4% methyl salicylate (methyl-2- hydroxybenzoate), 25% glycerin, and 5% polysorbate 80 in an aqueous solution. The ingredients are blended aggressively until a brilliant white emulsion forms.

Second experiment

In order to compare CMSE with clove powder, empirical doses of 526 μl/L CMSE and 150 mg/L clove powder used to anesthetize common carp.

Opercular rate (OpR)

Opercular rate as a function of concentration and time under anesthesia, was recorded during induction of anesthesia and recovery time.

Sampling and analyses

Blood was collected from the caudal vein of each fish using nonheparinized 3-mL syringes. Plasma was obtained after blood centrifugation (7 min, 5,000 rpm) at room temperature. The collected serum was frozen at -20 ˚C until plasma glucose analyses, using commercial kits (Pars Azmun Co. Ltd., Tehran, Iran), could be performed.

After adding the anesthetic agent, three fish was stocked into the each aquarium. In this study, induction time, recovery time, opercular rate, plasma glucose was noted and fish were maintained there for 72h in order to observe possible mortality.

Statistical analysis

Data were statistically analyzed by the SPSS 16 software. One-way analysis of variance (ANOVA) was employed to camper the means of factors. Where significant F-ratios were calculated by ANOVA, the Duncan test was applied to identify which means was different.

Results

The results obtained from the experiment are shown in Figures 1-3. All fish exposed to 263, 395, 526, 658 and 789 μI/L dose of CMSE at the experiment were anesthetized and recovered from anesthesia. No mortality occurred during the experiments.

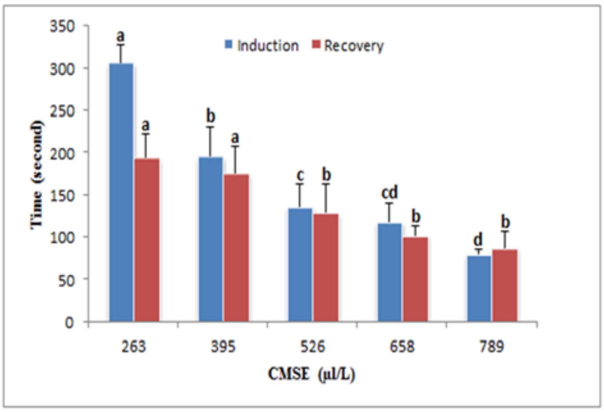

Figure 1: Induction and recovery time (mean ± SD) of common carp anesthetised with CMSE.

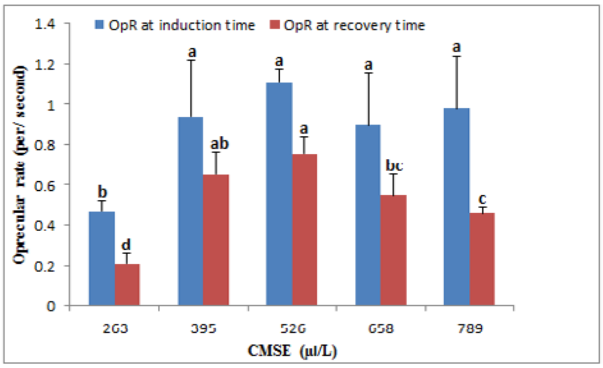

Figure 2: Opercular rate (OpR) at induction and recovery time of fish anesthetized with CMSE.

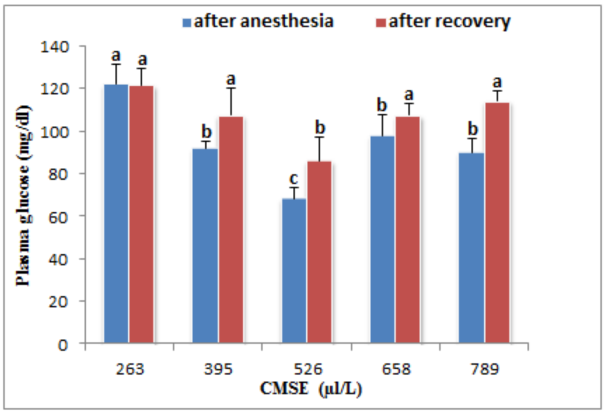

Figure 3: Glucose level profile of common carp after anesthesia and recovery.

In CMSE application, induction time for common carp changed between 80.33 ± 5.13-305.67 ± 21.13 second (Figure 1). There was significant difference in the induction time at the different concentrations of the CMSE tested (P<0.05). Behavioral anesthesia recovery occurred in <200s for all anesthetized fish (Figure 1). There was significant difference in the recovery time at the different concentrations of the CMSE tested (P<0.05).

According to the results, fish anesthetized with 263 μI/L showed a significant decrease in the induction opercular rate compared to other concentrations of the CMSE (P<0.05). The Highest of the opercular rate at induction and recovery time belong to concentration of 526 µl/L (Figure 2). There was significant difference in the opercular rate at recovery time (P<0.05).

In the case of plasma glucose after anesthesia and recovery, lower levels belong to treatment of 526 µl/L (Figure 3). The glucose levels were significantly affected by CMSE concentration (P<0.05). There was significant difference in the glucose levels after anesthesia and recovery under the different concentrations of the CMSE tested.

In the second experiment, fish anesthetized in CMSE reached stage 4 anesthesia in 137 s. Common carp anesthetized in clove powder reached 4 anesthesia in 157 s (Figure 4). However, common carp anesthetized in CMSE, had slower recovery time from stage 4 anesthesia than clove powder (Figure 4).

Common carp anesthetized in CMSE, had lowest opercular rate at induction and recovery time (Figure 5). Also, lower levels of plasma glucose after anesthesia and recovery belong to CMSE (Figure 6).

Discussion

In fish farming, anesthetics are necessary to lower the level of stress, to prevent any injuries of fish during handling such as particularly in artificial reproduction, marking, biometric evaluation etc [34]. Many factors affect the efficacy of anesthetics in fish. These factors can be divided into biological and environmental categories, and include species, body size, the density of the fish in a bath, and water quality. An ideal anesthetic for fish should induce anesthesia in less than 3 to 5 min, with total loss of equilibrium and muscle tone, should allow an uneventful and rapid (i.e. less than 10 min) recovery, should leave low tissue residues and be safe to users as well as be inexpensive and easy to use [35,36].

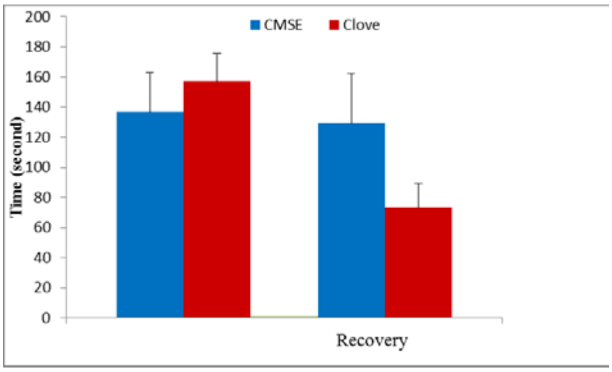

Figure 4: Anesthesia induction and recovery times for common carp anesthetized to stage 4 anesthesia with CMSE or clove powder.

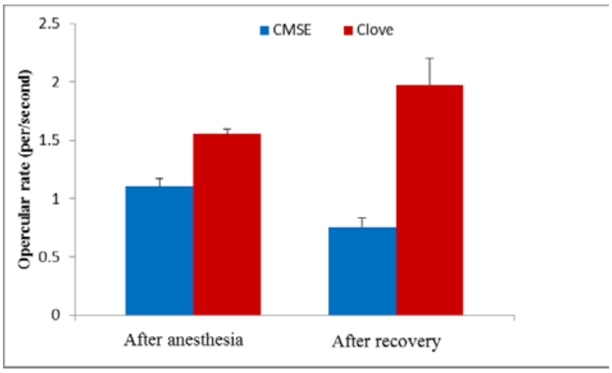

Figure 5: Opercular rate at induction and recovery times for common carp anesthetized to stage 4 anesthesia with CMSE or clove powder.

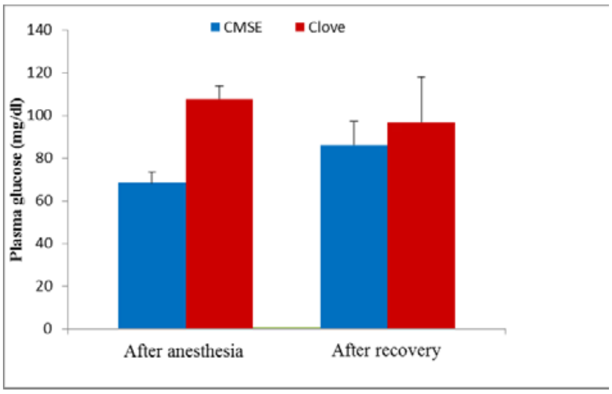

Figure 6: Plasma glucose at induction and recovery times for common carp anesthetized to stage 4 anesthesia with CMSE or clove powder.

In this study, we found CMSE to be an effective immersion fish anesthetic. An increasing concentration of the CMSE proportionally decreased the time required for anesthesia induction (Figure 1). This result is in accordance with previous findings in *Hippocampus reidi* exposed to the essential oil of *Lippia alba* [37] and *Cyprinus carpio* anesthetized with $NaHCO_3$ [38]. In our study, recovery time is usually faster at highest concentrations of anesthetic, and recovery time decrease with an increase in the concentration of the CMSE. This our result is consistent with previous findings in russian sturgeon [39] and *Rutlilus frissi kutum* [40] anesthetized with clove oil. This can indicate that induction time and recovery time of the fish can vary depending on concentration of anesthetic [36, 41]. Fish anesthetized with 789 µl/L concentration did not show any significant difference in the recovery time compared to 526 and 658 µl/L concentrations. So the 526 µl/L concentration is suggested as the optimal concentration for deep anesthesia induction.

Figure 2 shows the changes in the opercular rates of the common carp during anesthesia and recovery. Fish absorb anesthetic substances mainly through their gills [42]. Opercular rate firstly increased and then slowly decreased with increasing the concentration of anesthetic. This result is in accordance with previous findings in *Cyprinus carpio* anesthetized with NaHCO3 [38]. In our study, when fish were exposed to 526 µl/L of CMSE, highest opercular rates were observed during anesthesia or recovery. Different fish species have different opercular rates, which can reflect upon the rate of anesthetics absorption and upon anesthesia induction.

Smith [43] summarized reports on changes in plasma biochemical parameters due to fish stress. Based on his results, stress may decrease or increase blood glucose levels in different fish 1997; [44-46] In this study, significant differences in the plasma glucose levels after anesthesia and after recovery between the groups were observed (P<0.05). This our result is in accordance with findings of Mousavi [47]. The blood glucose levels of fish anaesthetized with 526 µl/L decreased significantly compared to other concentrations. Significant changes in glucose levels suggest that anesthesia with 526 µl/L dose of CMSE would be lower stress response compared to higher and lower doses.

According to the results obtained from the second experiment, CMSE had a slightly quicker induction time to stage 4 anesthesia than clove powder. The difference in induction time for stage 4 anesthesia between CMSE and MS-222 is not due to differences in fish size. Total body mass and length were normally distributed. However, CMSE had a slower recovery time from stage 4 anesthesia than clove powder (Figure 4). The opercular rate and glucose concentration of common carp was significantly reduced by CMSE and increased by clove powder. These findings demonstrate that CMSE has comparable induction and recovery times (stage 4 anesthesia) to clove powder.

In conclusion, the result of this study indicated that CMSE (526 µI/L) can induce safe and valid anesthesia in common carp that was higher (257 µI/L) than result of specie atlantic salmon (*Salmo salar sebago*) in study of Russell Danner et al. [48] So, CMSE applications can be a good alternative to other anesthetics.

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